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INDOLE ALKALOIDS AND TERPENOIDS FROM *TABERNAEMONTANA* MARKGRAFIANA

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Key Word Index—*Tabernaemontana markgrafiana*; Apocynaceae; bark; triterpenes; indole alkaloids.

Abstract—The bark of *Tabernaemontana markgrafiana* yielded five acetylated pentacyclic triterpenes and 24 monoterpene indole alkaloids. The major triterpene was baurenyl acetate, which constituted ca 6% of the crude petrol extract. An X-ray study of iso-ursenyl acetate was carried out for the first time. The indole alkaloids were primarily of the iboga-type and constituted ca 3% of the dried bark and 20% of the total extracts. The major alkaloids were coronaridine, (19S)-heyneanine, voacangine and ibogamine. Among the minor components, four new alkaloids were identified: 5,6-dehydro-coronaridine, 3R-methoxy-coronaridine, 3R-methoxyvoacangine and the 10,11-demethoxy chippline.

INTRODUCTION

Tabernaemontana markgrafiana, syn. *Bonafousia longituba* is a tree, growing sparsely in South America, giving a white latex. It is widely used in traditional medicine as a febrifuge and disinfectant in Brazil [1], as a contraceptive and against toothache in Peru, and as a fungicide, against toothache and insect bites in Ecuador [1, Ghia, unpublished results].

The genus *Tabernaemontana* has ca 120 species distributed in the tropical region and is characterized by its content of monoterpene indole alkaloids [2]. The genus is under revision by Leeuwenberg and has a large number of synonyms [1, 3]. The title species has not previously been investigated chemically. Extracts from the powdered bark showed a strong alkaloidal reaction with the Hager, Mayer, Dragendorff, Wanger and silicotungsten reagents.

RESULTS AND DISCUSSION

Since the alkaloid content in the bark was unusually high, the work was focused on this class of compounds; ca 3% of the dried bark and 20% of the total extracts consisted of alkaloids. ¹H NMR spectra of the crude alkaloidal fractions indicated the presence of indole alkaloids, which frequently occur in the genus *Tabernaemontana*. The plant material was successively extracted with petrol, dichloromethane and methanol. It was later established that the same alkaloids were present in both the dichloromethane and methanol fractions, and also to some extent in the petrol fraction. Therefore, the

work-up procedure could be simplified by just carrying out a petrol and a methanol extraction. Alkaloids were separated by extraction with 1% hydrochloric acid and basification with sodium hydrogen carbonate. The neutral petrol extract contained long-chain hydrocarbons (polyprenes), fatty acids, stigmaterol, α -amyrin acetate, β -amyrin acetate, baurenyl acetate 1a, iso-ursenyl acetate 2 and 20(30)-taraxasten-3 β -yl acetate 3a. The terpenes were identified by mp, $[\alpha]_D$, mass spectrometry, NMR, and by comparison with authentic samples. The last mentioned triterpene 3a had mp and $[\alpha]_D$ in closer agreement with 20(30)-ursen-3-yl acetate, 4 [4-6]. Hydrolysis of 3a gave the corresponding alcohol 3b, which by spectral comparison with an authentic sample was shown to be 20(30)-taraxasten-3-ol (taraxasterol), 3b [7] (Fig. 1).

Baurenyl acetate, 1a, precipitated as crystals during evaporation of the petrol extract. Its identity was confirmed by X-ray analysis. An X-ray determination of the structure has been carried out previously [8]. Basic hydrolysis yielded baurenol 1b. Baurenyl acetate was the major triterpene and constituted ca 6% of the crude petrol extract. A minor amount of another triterpene co-occurred with β -amyrin acetate. It could be purified by fractional recrystallization and an X-ray investigation proved it to be iso-ursenyl acetate 2 (Fig. 2). No new neutral compounds were isolated from the dichloromethane extract, which consisted mainly of alkaloids.

The alkaloids (Fig. 3) were represented primarily by iboga alkaloids, accounting for more than 90% of the isolated alkaloids (IA). A few per cent of the isolated alkaloids consisted of the aspidospermine-type alkaloids, O-acetylvallesamine 20 and vallesamine 21, and of the

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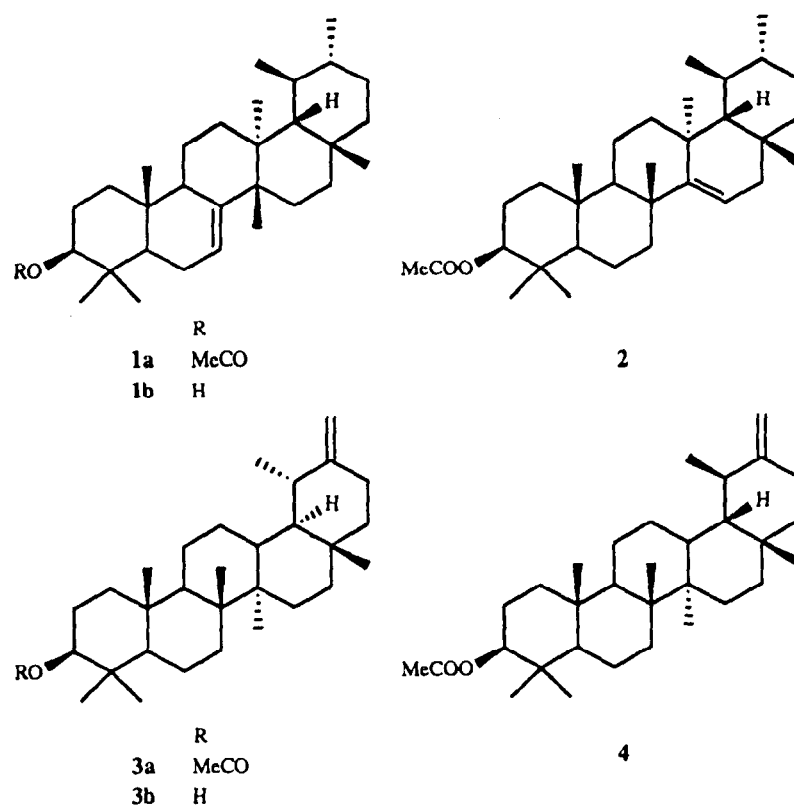
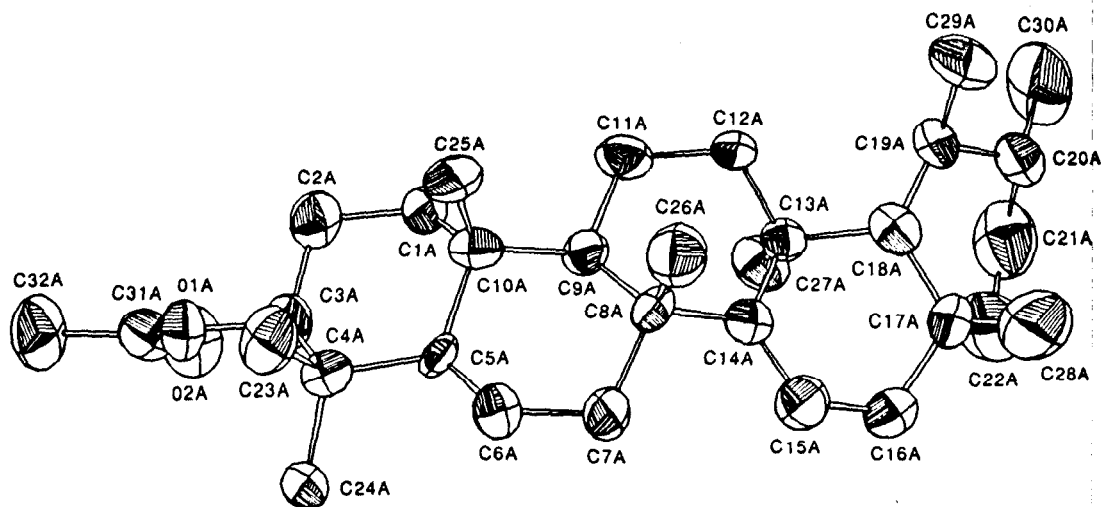


Fig. 1. Isolated triterpenoids (1-4).

Fig. 2. Perspective drawing of *iso*-urseny acetate (2), showing the atomic numbering.

corynantheine-type alkaloids, akuammidine 22 and 19*E*-16*R*-isositsirikine 23.

Coronaridine 5 (39% of IA), (19*S*)-heyneanine 7 (22%), voacangine 6 (9%), occurring in all extracts, and ibogamine 14 (8%) occurring in the dichloromethane and methanol extracts were the main alkaloids. The related alkaloids (19*S*)-voacristine 8 and ibogaine 15 isolated from the dichloromethane and methanol extracts consti-

tuted 4-5% of the alkaloid fraction. All the known alkaloids had physical data that agreed with those cited in the literature (see Experimental).

From the non-polar fraction of the dichloromethane extract a new unstable alkaloid was isolated, *M*, 336, i.e. 2 mu less than coronaridine 5. The ¹H NMR showed a *cis*-substituted double bond, but for the rest, most of the ¹H and ¹³C NMR characteristics of 5. Compound 10, i.e.

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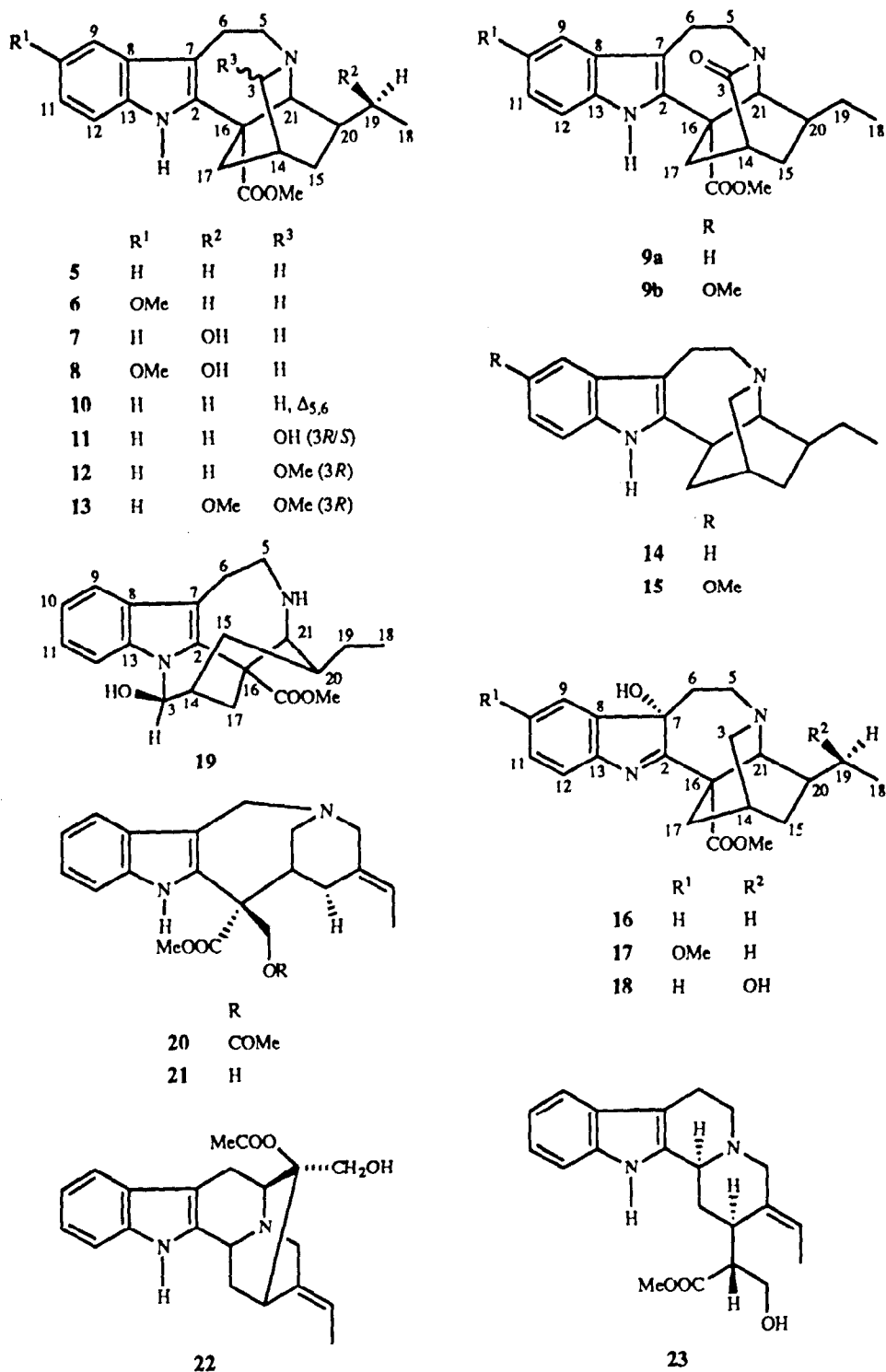


Fig. 3. Isolated alkaloids (5-23).

5,6-dehydro-coronaridine is therefore proposed for this alkaloid (0.3%).

The formation of the skeleton of the new alkaloid 19 (2%), is explained by hydrolysis of the (C-3)-(N-4) bond in 11 and recyclization involving the formation of the (N-

1)-(C-3) bond. One alkaloid, chippiine, with the same structural features has been reported previously [9]. Chippiine, is the 10,11-dimethoxyderivative of 19. The 15- α -H of 19 is located above the pyrrole ring, which explains its unusually low chemical shift of δ 0.32. The observed

chemical shift for H-3, δ 5.46, indicated a pseudo-axial orientation as shown in 19. The ^1H NMR spectrum of 19 is in close agreement with that of chipiline [9].

Secondary oxidations at C-3 and C-7 occur frequently in the iboga alkaloid series. The known compounds 3-oxocoronaridine 9a (0.6%), 3-oxovoacangine 9b (0.1%), 3*R/S*-hydroxycoronaridine, i.e. eglandine 11 (0.1%), and three 7-hydroxyindolenines 16, 17 and 18, 1.6, 1.5 and 0.1% of IA, were identified. From our 2D NMR measurements and Damak's data on 7 and 18 [10, 11], we have assigned the spectra of coronaridine hydroxyindolenine 16 and voacangine hydroxyindolenine 17 (see Experimental).

A minor alkaloid was isolated from the polar fractions. It contained two methyl groups, one at δ 3.7 (carbomethoxy), the other at δ 3.21 indicating an OMe-group of an N,O-acetal. The carbon atom at δ 96.4, correlating with a proton at δ 4.01, further confirmed the N,O-acetal structure at C-3 in an iboga alkaloid. Comparing the NMR spectra with data of 11 [12], and of 3*R/S*-ethoxycoronaridine [13], it became evident that the isolated compound was 3*R*-methoxycoronaridine 12. This alkaloid has not previously been described. It is not an artifact, since the petrol extract, in which it also occurred, had not been in contact with methanol. It was reported that 3-ethoxycoronaridine was isolated from *T. eglandulosa* and the authors stress that only solvents free from ethanol were used [13]. The 3*R*-configuration in 12 is proposed on the basis that the H-3*R* in the 3-hydroxy series is located at δ 4.1, while the H-3*S* is located at δ 4.4. The corresponding ^{13}C NMR shifts are δ 95 (C-3*R*) and δ 86 (C-3*S*) [9].

A small amount of 3*R*-methoxyvoacangine 13, occurring in the methanol extract, was also isolated and structurally determined by analogy to 12.

The four minor alkaloids, *O*-acetylvallesamine 20 (0.7%), vallesamine 21 (0.5%), 19(*E*)-akuammidine 22 (1.8%) and 19(*E*)-16*R*-isotsirikine 23 (0.8%), were isolated from the methanol extract and identified by comparing their spectral data with those cited in the literat-

ure. Moreover, the structure of 22 was confirmed by an X-ray study (Fig. 4).

EXPERIMENTAL

General. ^1H and ^{13}C NMR were recorded at 200 and 50 MHz, respectively, with TMS as int. standard. Units for chemical shifts are in ppm. For CC, Kieselgel 60, 63–200 μm , was used. Prep. TLC was carried out on glass plates 20 $\times$ 20 $\times$ 0.18 cm, on Kieselgel 60, $\text{P}_{254} + 366$ Merck. Mp are uncorr. Optical rotations were measured at 22–25°. Mass spectra were recorded at 70 eV, direct inlet.

Plant material. Bark of *T. markgrafiana* was collected in the Plantacion Forestal, Endesa, near the village of Pedro Vicente de Maldonado, Provincia de Pichincha, Ecuador at an altitude of 750 m. Voucher specimens, FG 475, are deposited at the Escuela Politécnica Nacional, Quito. The bark was dried in a ventilated hood at ca 45° in the dark.

Extraction. Dried and ground bark (1.24 kg) was extracted at 25° in the dark with petrol (3 $\times$ 4 l), CH_2Cl_2 (3 $\times$ 4 l) and MeOH (3 $\times$ 4 l). Extracts were evapd *in vacuo* to give 48, 39 and 97 g, respectively.

The petrol extract yielded a ppt., which by recrystallization in CH_2Cl_2 , gave crystals of *baurenol acetate* (1a), 3 g. Glistening plates (CH_2Cl_2). Mp 294–298° (lit. 292–294° [14], 282–284° [15]). $[\alpha]_D - 2.3^\circ$ (CHCl_3 ; c 1.02) (lit. -2.5° [15]). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1735, 1255, 821. EIMS m/z : 468 $[\text{M}]^+$, 408, 393, 229. ^{13}C NMR (CDCl_3): δ 12.8, 15.6, 16.6, 21.2, 22.4, 22.5, 23.5, 23.8, 24.0, 25.5, 27.3, 28.7, 29.0, 31.4, 31.9 (2 $\times$ C), 32.2, 34.9, 35.2, 36.4, 37.55, 37.59 (2 $\times$ C), 37.9, 41.1, 48.0, 50.5, 54.8, 81.1, 116.3, 145.6, 171.3. Basic hydrolysis of 1a afforded *baurenol* (1b) as needles (EtOH), Mp 203–209° (lit. 208–210° [15]). $[\alpha]_D - 22.3^\circ$ (CHCl_3 ; c 0.89), (lit. -18° [15]). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3430, 821. EIMS m/z : 426 $[\text{M}]^+$, 408, 229. ^{13}C NMR (CDCl_3): δ 12.8, 14.5, 16.6, 22.4, 22.5, 23.5, 24.0, 25.5, 27.4, 27.5, 28.7, 29.0, 31.3,

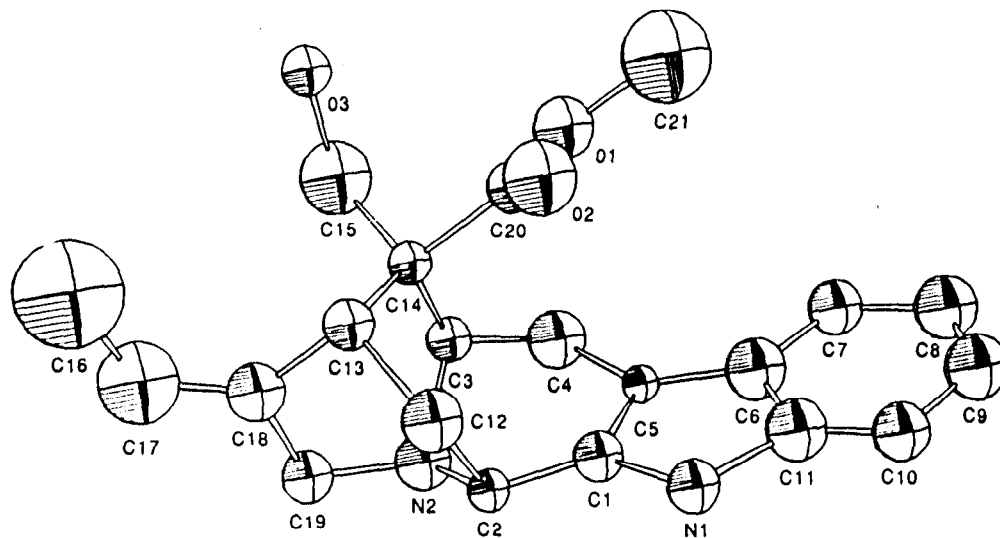


Fig. 4. Perspective drawing of 19(*E*)-akuammidine (22), showing the atomic numbering.

31.9 (2 $\times$ C), 48.1, 50.3, 54.8. The petrol neutral fr. (39 g) was chromatographed on increasing amounts of silica gel (0.14 g), 16 (0.04 g), 12 (0.03 g) in order of purification. The alk. (50%) despoiled not possible 'neutral fr.' in the neutral. Crude (17 g) were petrol- CH_2Cl_2 , followed by CH_2Cl_2 -MeOH (5 g) was purified by EtOAc-MeOH (8 [21, 26, 15 [11, 20]. The M. EtOAc (2. of soluble fr. (1.5 g) trum of t extract.

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31.9 (2 × C), 32.2, 35.0, 35.2, 36.7, 37.5, 37.9, 38.7, 41.1, 48.1, 50.3, 54.8, 58.3, 79.2, 116.5, 145.5.

The petrol filtrate was extracted with 1% HCl to give a neutral fr. (39 g) and an alkaloid fr. (2.4 g). The neutral fr. was chromatographed on silica gel with petrol containing increasing amounts of EtOAc as eluent. The non-polar frs contained satd hydrocarbons and polyisoprenes. By TLC of the terpenoid frs, fractional recrystallization, comparison with authentic samples and lit. NMR data, α - and β -amyrin acetate, stigmasterol, iso-ursenyl acetate 2 (X-ray analysis, Fig. 1) and 20(30)-taraxasten-3-yl acetate 3a [16] were identified. Basic hydrolysis of 3a afforded taraxasterol 3b [7, 18, 19] as thin needles. The more polar frs contained fatty acids.

iso-Ursenyl acetate (2). Transparent needles (EtOAc). Mp 196–200° (lit. 214–216° [16], 213° [17]). ¹H NMR (CDCl₃): δ 0.85 (3H, s), 0.93 (6H, s), 0.93 (6H, s), 0.99 (3H, s), 1.06 (3H, s), 4.46 (1H, m), 5.49 (1H, dd, J = 3 and 8 Hz). ¹³C NMR (CDCl₃): δ 15.1, 16.4, 17.3, 18.6, 19.2, 22.3, 23.4, 26.2, 27.3, 27.8, 28.3, 32.0, 33.7, 35.3, 36.4, 37.0, 37.2, 37.5, 37.7, 38.3, 38.8, 39.9, 40.5, 41.7, 49.0, 55.4, 60.4, 81.0, 116.5, 159.6, 171.3.

The alkaloids of the petrol fr. were chromatographed on a silica gel column with petrol containing increasing amounts of EtOAc to give 5 [2, 11] (1.3 g), 6 [11, 21] (0.14 g), 16 [20] (0.01 g), 17 [20, 22] (0.06 g), 9a [12, 23] (0.04 g), 12 (0.02 g) and 7 [2, 10, 11, 20, 21] (0.07 g), eluted in order of increasing polarity of the mobile phase. Purification of frs was carried out by TLC with petrol–Et₂O and CHCl₃–MeOH as solvent systems.

The alkaloid content of the CH₂Cl₂ extract was ca 50%. Despite repeated extraction with 1% HCl, it was not possible to completely remove all alkaloids. The 'neutral fr.' contained similar compounds to those found in the neutral petrol fr. No new compounds were detected. Crude alkaloids (8.6 g) from the CH₂Cl₂ extract (total 17 g) were sepd on a silica gel column starting with petrol–CH₂Cl₂ (1:1) and increasing amounts of CH₂Cl₂, followed by CH₂Cl₂–MeOH mixts. 5,6-Dehydrocoronaridine 10 (33 mg) occurred in the early frs. It was followed by 5 (2.32 g), 6 (0.37 g), 7 (0.4 g), 16 (0.03 g), 17 (0.03 g) and 18 [10] (0.01 g). The major part of the alkaloids (5 g) was eluted as a narrow band with CH₂Cl₂–MeOH (20:1). From a fr. at the end of the band, a small amount of O-acetyl-vallesamine 20 [24, 25] (0.05 g) was isolated. The last frs, which were eluted with CH₂Cl₂–MeOH (5:1), contained several minor polar alkaloids which were not studied further. The major fr. (5 g) was rechromatographed on silica gel with a petrol–EtOAc–MeOH system and the frs obtained were further purified by TLC on silica gel to give 6 (66 mg), 7 (790 mg), 8 [21, 26, 27] (135 mg), 12 (70 mg), 14 [2, 11, 20] (324 mg), 15 [11, 20] (219 mg) and small amounts of 16 and 17.

The MeOH extract (97 g) was extracted × 2 with EtOAc (250 ml) for 1 day with stirring at 25°, to give 7.1 g of soluble material, which was partitioned into a neutral fr. (1.5 g) and an alkaloid fr. (3.4 g). The ¹H NMR spectrum of these crude alkaloids was identical to the spectrum of the crude alkaloids obtained from the CH₂Cl₂ extract. Therefore, the work was concd on the basic

constituents of the solid residue (85 g), poorly soluble in EtOAc. It was finely divided in a mortar and 36 g of the pulverized extract was extracted with HCl (8 × 100 ml), with stirring at 25°. Filtration left a solid residue of 9 g, which was discarded. The filtrate was basified with NaHCO₃ and extracted with EtOAc. The emulsion was filtered, the organic phase sepd and evapd to give 4.8 g alkaloids. The EtOAc-insoluble ppt. (ca 1 g) also showed a strong alkaloid reaction, but was not further investigated. The alkaloid fr. (4.8 g) was chromatographed on a silica gel column with CHCl₃–MeOH (97:3) and increasing amounts of MeOH. The combined frs were rechromatographed on another column by gradient elution with petrol–Et₂O–MeOH. Further purification was carried out by TLC using CHCl₃–MeOH, Et₂O–MeOH–NH₃, toluene–EtOAc–NH₃ and toluene–EtOH–NH₃. An NH₃ atm. was obtained by placing a flask containing NH₃ (aq.) in the chamber. The TLC plates were satd 20 min prior to development. The following alkaloids were isolated with increasing polarity of the mobile phase: 5 (360 mg), 6 (156 mg), 11 [12] (5 mg), 16 (16 mg), 17 (9 mg), 7 (433 mg), 14 (192 mg), 12 (75 mg), 13 (24 mg), 9a (40 mg), 9b [22] (11 mg), 8 (159 mg), 15 (143 mg), 20 (28 mg), 19 (60 mg), 21 [24, 25, 28] (19 mg), 22 (47 mg) [26, 28–30] and 23 [31, 32] (16 mg). Additionally a vallesamine isomer was isolated (18 mg), the absolute configuration of which was not elucidated.

5,6-Dehydrocoronaridine (10). Amorphous reddish-white material which decomposed in CHCl₃. EIMS m/z : 336 [M]⁺. ¹H NMR (CDCl₃–CCl₄, 3:2): δ 0.86 (3H, t, J = 7 Hz, H-18), 1.15 (1H, dm, J = 12 Hz, H-15), 1.2–1.85 (6H, m), 2.62 (1H, br d, J = 9 Hz, H-3), 3.00 (1H, d, J < 0.5 Hz, H-21), 3.01 (1H, dm, J = 12 Hz, H-17), 3.36 (1H, br d, J = 9 Hz, H-3), 3.70 (3H, s, COOMe), 6.12 (1H, d, J = 7.5 Hz, H-6), 6.27 (1H, d, J = 7.5 Hz, H-5), 7.03–7.18 (2H, m), 7.22 (1H, br d, J ~ 7.5 Hz), 7.62 (1H, br d, J ~ 7.5 Hz), 7.87 (1H, br s, NH). ¹³C NMR (CDCl₃–CCl₄, 3:2): δ 12.2, 22.6, 28.8, 30.3, 36.1, 37.2, 52.7, 53.5, 54.5 (2 × C), 106.3, 110.2, 110.8, 118.9, 120.1, 122.6, 127.8, 135.6, 137.1, 137.4, 175.0.

(–)-3R-Methoxycoronaridine (12). Amorphous light yellow solid. [α]_D –44° (CHCl₃; c 0.3). IR $\nu_{\max}^{\text{film}}$ cm^{–1}: 3360, 3260, 1720, 1660, 1455. EIMS m/z (rel. int.): 368 [M]⁺ (3), 367 (2, [M – 1]⁺), 366 (4, [M – 2]⁺), 353 (5, [M – 15]⁺), 352 (18, [M – 16]⁺), 338 (54), 336 (66), 323 (15), 307 (12), 278 (11), 277 (27). ¹H NMR (CDCl₃): δ 0.92 (3H, t, J = 7 Hz, H-18), 1.32 (1H, m, H-20), 1.45–1.65 (5H, m, H-5, 5', 15, 19, 19'), 1.93 (1H, dd, J = 13.5 and 4 Hz), 2.05 (1H, br m, H-14), 2.79 (1H, dd, J = 13.5 and 2 Hz, H-17'), 3.11 (2H, m, H-6, 6'), 3.21 (3H, s, OMe), 3.70 (3H, s, COOMe), 4.01 (1H, d, J = 2 Hz, H-3), 7.10 (1H, br dd, J = 7 Hz, H-10), 7.17 (1H, br dd, J = 7 Hz, H-11), 7.28 (1H, br d, J = 7 Hz, H-12), 7.51 (1H, br d, J = 7 Hz, H-9), 7.9 (1H, br s, NH). ¹³C NMR (CDCl₃): δ 12.2 (q, C-18), 22.3 (t, C-6), 25.5 (t, C-15), 27.1 (t, C-19), 30.5 (d, C-14), 35.9 (t, C-17), 38.3 (d, C-20), 53.1 (t, C-5), 53.2 (q, COOMe), 54.5 (q, OMe), 54.8 (s, C-16), 56.0 (d, C-21), 96.4 (d, C-3), 110.6 (s, C-7), 111.0 (d, C-12), 118.8 (d, C-9), 119.8 (d, C-10), 122.4 (d, C-11), 128.7 (s, C-8), 136.0 (s, C-13), 136.9 (s, C-2), 175.4 (s, COOMe).

(-)-3R-Methoxyvoacangine (13). Amorphous light green solid. $[\alpha]_D - 39^\circ$ (CHCl₃; c 0.4). EIMS m/z (rel. int.): 398 [M]⁺ (2.3), 382 (12), 369 (8), 368 (34), 367 (34), 366 (100), 365 (16), 351 (17), 337 (21), 308 (21), 307 (33), 283 (16), 279 (20), 268 (26), 267 (12), 258 (17), 244 (18), 225 (22), 184 (17), 153 (13), 136 (33), 135 (22), 124 (18), 122 (24). ¹H NMR (CDCl₃): δ 0.91 (3H, t, $J = 7.4$ Hz, H-18), 1.35 (1H, m, H-20), 1.5–1.6 (5H, m, H-5,5',15,19,19'), 1.90 (1H, dd, $J = 13.5$ and 3.5 Hz, H-17), 2.04 (1H, br s, H-14), 2.79 (1H, dd, $J = 13.5$ and 2.3 Hz, H-17'), 3.07 (2H, m, H-6,6'), 3.21 (3H, s, OMe), 3.70 (3H, s, COOMe), 3.86 (3H, s, ArOMe), 4.02 (1H, d, $J = 2.4$ Hz, H-3S), 6.82 (1H, dd, $J = 8.6$ and 3.4 Hz, H-11), 6.95 (1H, d, $J = 3.4$ Hz, H-9), 7.16 (1H, d, $J = 8.6$ Hz, H-12), 7.77 (1H, (1H, br s, NH). ¹³C NMR (CDCl₃): δ 12.2 (C-18), 22.4 (C-6), 25.4 (C-15), 27.1 (C-19), 30.4 (C-14), 35.8 (C-17), 38.3 (C-20), 53.0 (C-5), 53.2 (COOMe), 54.5 (OCMe), 54.9 (C-16), 56.0 (C-21), 56.5 (ArOMe), 96.4 (C-3), 101.0 (C-9), 110.4 (C-7), 111.7 (C-12), 112.4 (C-11), 128.1 (C-8), 131.1 (C-13), 137.9 (C-2), 154.6 (C-10), 175.4 (COOMe).

Coronaridine hydroxyindolenine (16). Amorphous light brown solid. $[\alpha]_D - 8^\circ$ (CHCl₃; c 1.04). ¹H NMR (CDCl₃): δ 0.86 (3H, t, $J = 7.5$ Hz, H-18), 1.14 (1H, br dd, $J = 12$ and 5 Hz, H-15), 1.36 (1H, m, H-20), 1.44 (2H, m, H-19, 19'), 1.73 (1H, m, H-15'), 1.86 (1H, m, H-6), 1.90 (1H, m, H-14), 1.97 (1H, m, H-6'), 2.46 (1H, ddd, $J = 14.4$ and 3 Hz, H-17), 2.67 (1H, m, H-17'), 2.71 (2H, m, H-3), 2.96 (1H, ddd, $J = 15.4$ and 2 Hz, H-5), 3.47 (1H, ddd, $J = 15.12$ and 4 Hz, H-5'), 3.68 (3H, s, COOMe), 3.79 (1H, br s, H-21), 7.17–7.38 (3H, m, H-9,11,12), 7.44 (1H, br d, $J = 7$ Hz, H-10). ¹³C NMR (CDCl₃): δ 12.1 (q, C-18), 27.0 (t, C-19), 27.5 (d, C-14), 32.5 (t, C-15), 34.3 (t, C-6), 35.3 (t, C-17), 38.0 (d, C-20), 49.2 (t, C-3), 49.6 (t, C-5), 53.7 (q, COOMe), 58.9 (d, C-21), 59.0 (s, C-16), 88.8 (s, C-7), 121.3 (d, C-10), 121.7 (d, C-12), 127.3 (d, C-9), 129.9 (d, C-11), 143.1 (s, C-8), 151.8 (s, C-13), 189.7 (s, C-2).

Voacangine hydroxyindolenine (17). Amorphous yellow-green solid. $[\alpha]_D - 26^\circ$ (CHCl₃; c 1.1) (lit. $[\alpha]_D - 23.1^\circ$ [33], -18° [20]). EIMS m/z : 384 [M]⁺, 383, 367, 355, 325, 260, 218, 190, 176, 162, 160. ¹H NMR (CDCl₃): δ 0.87 (3H, t, $J = 7$ Hz, H-18), 1.10 (1H, br dd, $J = 12$ and 5 Hz, H-15), 1.35–1.48 (3H, m, H-19, 20), 1.63 (1H, br s, OH), 1.7–1.95 (4H, m, H-6,14,15), 2.47 (1H, ddd, $J = 14.5$ and 2 Hz, H-17), 2.71 (1H, dm, $J = 14$ Hz, H-17'), 2.74 (2H, m, H-3), 2.97 (1H, ddd, $J = 15.4$ and 2 Hz, H-5), 3.5 (1H, ddd, $J = 15.11$ and 4 Hz, H-5'), 3.61 (1H, d, $J = 1.8$ Hz), 3.71 (3H, s, COOMe), 3.77 (1H, s, H-21), 3.83 (3H, s, ArOMe), 6.81 (1H, dd, $J = 8.5$ and 2.5 Hz, H-11), 6.92 (1H, d, $J = 2.5$ Hz, H-9), 7.37 (1H, d, $J = 8.5$ Hz, H-12). ¹³C NMR (CDCl₃): δ 11.7 (q, C-18), 26.7 (t, C-19), 27.2 (d, C-14), 32.3 (t, C-15), 34.4 (t, C-6), 34.7 (t, C-17), 37.8 (d, C-20), 48.9 (t, C-3), 49.4 (t, C-5), 53.5 (q, COOMe), 56.0 (q, ArOMe), 58.8 (d, C-21), 58.9 (s, C-16), 88.7 (s, C-7), 108.4 (d, C-9), 114.2 (d, C-11), 121.8 (d, C-11), 121.8 (d, C-12), 145.0* (s, C-8), 145.4* (s, C-13), 159.7 (s, C-10), 187.5 (s, C-2).

Alkaloid (19). Brown oil. $[\alpha]_D - 26^\circ$ (CHCl₃; c 0.02). IR $\nu_{\max}^{\text{film}}$ cm⁻¹: 3370, 1725, 1470. EIMS m/z (rel. int.): 355 (5), 354 [M]⁺ (21), 338 (9), 337 (10), 336 [M-H₂O]⁺ (24), 238 (8), 180 (7), 169 (8), 168 (6), 167 (8), 154 (9), 143 (7), 136

(5), 135 (5), 86 (70), 84 (100). ¹H NMR (300 MHz, CDCl₃): δ 0.32 (1H, ddd, $J = 14.2, 14.1$ and 7.1 Hz, H-15 α), 0.91 (3H, t, $J = 7$ Hz, H-18), 1.00 (1H, dqd, $J = 12.9, 6.9$ and 7.1 Hz, H-19), 1.39 (1H, dqd, $J = 12.9, 6.9$ and 7.1 Hz, H-19'), 1.62 (1H, ddd, $J = 14.2, 10.5$ and 3.4 Hz, H-15 β), 1.84 (1H, dddd, $J = 14.0, 6.9, 6.9, 6.9$ and 3.4 Hz, H-20), 2.02 (1H, dd, $J = 13.4$ and 5 Hz, H-17 β), 2.34 (1H, dd, $J = 13.4$ and 1 Hz, H-17 α), 2.49 (1H, m, H-14), 2.67 (2H, m, H-6,6'), 2.95 (1H, m, H-5 β), 3.19 (1H, m, H-5 α), 3.70 (3H, s, COOMe), 3.93 (1H, d, $J = 6.9$ Hz, H-21), 5.46 (1H, d, $J = 2.2$ Hz, H-3), 7.16 (1H, ddd, $J = 7.1, 7.1$ and 1.1 Hz, H-10), 7.22 (1H, ddd, $J = 7.1, 7.1$ and 1.1 Hz, H-11), 7.47 (1H, dd, $J = 7.1$ and 1.1 Hz, H-12), 7.50 (1H, dd, $J = 7.1$ and 1.1 Hz, H-9). ¹³C NMR (50 MHz, CDCl₃): δ 12.9 (q, C-18), 23.5 (t, C-15), 24.4 (t, C-17), 24.5 (t, C-6), 24.7 (t, C-19), 34.9 (d, C-14), 36.4 (d, C-20), 40.9 (t, C-5), 49.7 (s, C-16), 53.2 (q, OMe), 58.8 (d, C-21), 80.0 (d, C-3), 109.9 (d, C-12), 110.1 (s, C-7), 118.8 (d, C-9), 120.9 (d, C-10), 122.2 (d, C-11), 129.5 (s, C-8), 132.0 (s, C-8), 132.0 (s, C-13), 137.7 (s, C-2), 176.7 (s, C=O).

19E-16R-Isositsirikine (23). Brown oil. ¹³C NMR (CDCl₃): δ 13.9, 18.1, 30.1, 33.0, 49.9, 51.3, 52.8, 53.2, 61.0, 62.4, 107.9, 111.9, 118.5, 120.1, 122.2, 125.4, 127.8, 133.0, 133.2, 136.8, 175.7.

Vallesamine isomer. $[\alpha]_D + 80^\circ$ (MeOH; c 0.8) (lit. 19(E)-val: $[\alpha]_D + 165^\circ$ (CHCl₃) [25, 28], 19(Z)-val: $[\alpha]_D + 182^\circ$ (CHCl₃) [28]). EIMS m/z (rel. int.): 354 (19), 340 (14, [M]⁺), 339 (17, [M-1]⁺), 310 (32, [M-30]⁺), 309 ([M-31]⁺), 215 (25), 208 (18), 202 (19), 201 (15), 194 (17), 124 (24), 123 (28), 122 (100). ¹H NMR (CDCl₃): δ 1.73 (3H, d, $J = 7$ Hz, H-18), 2.16 (1H, m, H-14), 2.22 (1H, m, H-14'), 3.00–3.30 (2H, m, H-3, 3'), 3.60 (2H, m, H-21, 21'), 3.66 (1H, m, H-15), 3.75 (3H, s, COOMe), 3.85 (1H, d, $J = 10.5$ Hz, H-17), 4.08 (1H, d, $J = 16.2$ Hz, H-6), 4.22 (1H, d, $J = 10.5$ Hz, H-17'), 5.09 (1H, d, $J = 16.2$ Hz, H-6'), 5.58 (1H, q, $J = 7$ Hz, H-19), 7.10 (1H, t, $J = 7$ Hz, H-11), 7.20 (1H, t, $J = 7$ Hz, H-10), 7.34 (1H, br d, $J = 7.7$ Hz, H-12), 7.49 (1H, br d, $J = 7.8$ Hz, H-9), 10.34 (1H, br s, NH). ¹³C NMR (CDCl₃): δ 14.8 (C-18), 21.2 (C-14), 35.2 (C-15), 47.9 (C-3), 49.1 (C-6), 52.3 (OMe), 53.8 (C-21), 58.9 (C-16), 70.5 (C-17), 102.4 (C-7), 111.7 (C-12), 120.6 (C-10), 123.6 (C-11), 125.7 (C-19), 127.8 (C-8), 130.5 (C-20), 135.3 and 135.3 (C-2 and C-13), 173.8 (C=O).

X-ray data for 1a, 2 and 22. Crystals were mounted on a Huber diffractometer. Unit cell dimensions were determined from the setting angles of reflections measured at $\pm\theta$ and at high and low χ with graphite monochromated Mo K α ($\lambda = 0.71073$ Å) radiation.

Crystal data. Compound 1a, C₃₂H₄₉O₂, $M_r = 465.74$, orthorhombic space group P2₁2₁2₁, $a = 11.084(9)$, $b = 8.294(5)$, $c = 30.040(17)$ Å; $V = 2764(3)$ Å³ from 80 reflections; $Z = 4$, $D_c = 1.119$ g cm⁻³; $\mu(\text{Mo}) = 0.63$ cm⁻¹; $T = 294$ K. Compound 2, C₃₂H₅₂O₂, $M_r = 468.77$, monoclinic space group P2₁, $a = 14.713(2)$, $b = 7.381(2)$, $c = 26.079(5)$ Å, $\beta = 96.048(8)^\circ$ from 100 reflections; $V = 2816.4(9)$ Å³; $Z = 4$, $D_c = 1.105$ g cm⁻³; $\mu(\text{Mo}) = 0.62$ cm⁻¹; $T = 294$ K. Compound 22, C₂₁H₂₄N₂O₃·CHCl₃, $M_r = 471.83$, orthorhombic space group P2₁2₁2₁, $a = 6.311(3)$, $b = 13.450(9)$, $c = 28.501(17)$ Å; $V = 2419(2)$ Å³ from 120 reflections; z

$= 4$, $D_c = 1.29$. Data were collected on a Siemens Kristalloflex D5000 X-ray diffractometer. For 1a, 25 were measured with a 0.14 mm. For 2, 1371 with $0.14 \times 0.14 \times 0.14$ mm. For 22, 1371 with $0.14 \times 0.14 \times 0.14$ mm. CHCl₃ was lost from the first small data set which 819 with

Structures were solved by direct methods and refined by least-squares on F^2 . All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were included at calculated positions with $U = 0.050$ and $wR = 0.057$ for 22. In 2, the asymmetry of the molecule was taken into account by the mole

[34]. Fractional coordinates and atom labels are given in the Crystallographic Data Tables.

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1. Van B.
2. A. Lex
3. J. Eth
4. Danie
5. 27, 1.
6. Van I
7. Front
8. (Dor)
9. Teslo
10. Nat.
11. Pano
12. Khin
13. trans
14. Lian
15. Cher
16. Patr
17. (198
18. Tina

$= 4$, $D_c = 1.295 \text{ g cm}^{-3}$, $\mu(\text{Mo}) = 4.03 \text{ cm}^{-1}$; $T = 294 \text{ K}$. Data were collected with the $\omega - 2\theta$ scan technique, $20 \leq 2\theta \leq 48.0^\circ$, reflections were corrected for background, Lorentz and polarization effects, and for absorption. For **1a**, 2582 unique reflections, 1181 with $I > 3\sigma(I)$, were measured from a crystal of dimensions $0.70 \times 0.70 \times 0.14 \text{ mm}$. For **2**, 4815 unique reflections 1851 with $I > 3\sigma(I)$, were measured from a crystal of dimensions $0.16 \times 0.45 \times 0.20 \text{ mm}$. For **22**, 2491 unique reflections, 1371 with $I > 3\sigma(I)$, were measured from a crystal of dimensions $0.55 \times 0.25 \times 0.04 \text{ mm}$. During data collection CHCl_3 was lost and intensities fell off by nearly 50%. The first small data set of 1345 reflections with $2\theta \leq 40.0^\circ$, of which 819 with $I > \sigma(I)$ were used in the final refinements.

Structures were solved by direct methods using SHELX-86 on a VAX 6210 computer and refined by the least-squares minimization of $\sum w(|F_o| - |F_c|)^2$. Hydrogen atoms were located on a difference map, but were included at calc'd positions ($\text{C-H} = 0.95 \text{ \AA}$), the methyl groups were disordered in **2**. The final R -values were $R_F = 0.050$ and $wR(F) = 0.058$ for **1a**, $R(F) = 0.050$ and $wR(F) = 0.057$ for **2**, and $R(F) = 0.164$ and $wR(F) = 0.158$ for **22**. In **2**, there are two almost identical molecules in the asymmetric unit. The crystals of **22** contained solvent, but the molecular structure found is similar to that in ref. [34].

Fractional coordinates, thermal parameters, bond distances and angles have been deposited at the Cambridge Crystallographic Data Centre.

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REFERENCES

- Van Beek, T. A., Verpoorte, R., Baerheim Svendsen, A., Leeuwenberg, A. J. M. and Bisset, N. G. (1984) *J. Ethnopharm.* **10**, 1.
- Danieli, B. and Palmisano, G. (1986) *The Alkaloids* **27**, 1.
- Van Beek, T. A. (1990) in *Phytochemistry, Methods, Frontiers*, Vol. 1. Suppl. Rev. Latinoam. Quim. (Dominguez, S. X. A., ed.), p. 270. Mexico.
- Teslov, L. S. (1984) *Khim. Prir. Soedin.* **20**, 665; *Chem. Nat. Compd* (Eng. transl.) 635.
- Panosyan, A. G. and Mnatsakanyan, V. A. (1977) *Khim. Prir. soedin.* **13**, 59; *Chem. Nat. Compd* (Eng. transl.) 50.
- Liang, J. and Chen, Y. (1982) *Zhongcaoyao* **13**, 8; *Chem. Abstr.* **97**, 212 634n.
- Patra, A., Mukhopadhyay, A. K. and Mitra, A. K. (1981) *Org. Mag. Reson.* **17**, 166.
- Tinant, B., Germain, G., Declercq, J. P., Van Meerssche, M., Ciccio, J. F. and Hoet, P. (1982) *Bull. Soc. Chim. Belg.* **91**, 117.
- Van Beek, T. A., Verpoorte, R. and Baerheim Svendsen, A. (1985) *J. Nat. Prod.* **48**, 400.
- Damak, M. (1977) *Analyse structurale et conformationnelle d'alkaloides isolés de Bonafousia tetrastachya*, Thesis, l'Universite de Paris-Sud Centre d'Orsay, France.
- Damak, M., Poupat, C. and Ahond, A. (1976) *Tetrahedron Letters* **39**, 3531.
- Le Men-Olivier, L., Le Men, J., Massiot, G., Richard, B., Mulamba, T., Potier, P., Husson, H.-P., Van Beek, T. A. and Verpoorte, R. (1985) *Bull. Soc. Chim. Fr.* **2**, 94.
- Achenbach, H. and Raffelsberger, B. (1980) *Phytochemistry* **19**, 716.
- Prager, R. H. and Thredgold, H. M. (1966) *Aust. J. Chem.* **451**.
- Row, L. R., Rao, C. S. and Ramaiah, T. S. (1969) *Ind. J. Chem.* **7**, 204.
- Dominguez, X. A., Marroquin, J. and Gutierrez, M. (1975) *Phytochemistry* **14**, 815.
- Chivers, H., Corbett, R. E. and Mitchell, R. E. M. (1966) *J. Chem. Soc. (C)* 1814.
- Ames, T. R., Beton, J. L., Bowers, A., Halsall, T. G. and Jones, E. R. H. (1954) *J. Chem. Soc.* 1905.
- Arthur, H. R. and Ko, P. D. S. (1969) *Aust. J. Chem.* **22**, 597.
- Achenbach, H. and Raffelsberger, B. (1980) *Z. Naturforsch.* **35b**, 219.
- Gunasekera, S. P., Cordell, G. A. and Farnsworth, N. R. (1980) *Phytochemistry* **19**, 1213.
- Agwada, V. C., Morita, Y., Renner, U., Hesse, M. and Schmid, H. (1975) *Helv. Chim. Acta* **58**, 1001.
- Feng, X. Z., Khan, C., Potier, P., Kan, S.-K. and Lounasmaa, M. (1982) *Planta Med.* **44**, 212.
- Perera, P., Sandberg, F., Van Beek, T. A. and Verpoorte, R. (1984) *Planta Med.* **251**.
- Walser, A. and Djerassi, C. (1964) *Helv. Chim. Acta* **47**, 2072.
- Achenbach, H. and Raffelsberger, B. (1980) *Z. Naturforsch.* **35b**, 885.
- Perera, P., Samuelsson, G., Van Beek, T. A. and Verpoorte, R. (1983) *Planta Med.* **47**, 148.
- Atta-ur-Raman, Alvi, K. A., Abbas, S. A. and Voelter, W. (1987) *Heterocycles* **26**, 413.
- Yagudaev, M. R. (1986) *Chem. Nat. Compd. Uzbek SSR Acad. Sci.* (Eng. transl.) **22**, 1.
- Lounasmaa, M., Jokela, R., Tolvanen, A. and Kan, S.-K. (1985) *Planta Med.* **519**.
- Kan, C., Kan, S.-K., Lounasmaa, M. and Husson, H.-P. (1981) *Acta Chem. Scand. B* **35**, 269.
- Kutney, J. P. and Brown, R. T. (1966) *Tetrahedron* **22**, 321.
- Gower, A. E., Pereira, B. da S. and Marsaioli, A. J. (1986) *Phytochemistry* **25**, 2908.
- Ponglux, D., Wongseripipatana, S., Subhadhirasakul, S., Takayama, H., Yokota, M., Ogata, K., Phisalaphong, C., Aimi, N. and Sakai, S. (1988) *Tetrahedron* **44**, 5075.